Biomedical Applications of Nanodiamonds in Microbiology

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DOI:
10.33612/diss.250888650

Publication date:
2022

Summary

In the past years, nanodiamonds (NDs) have attracted increasing attention due to their unique properties and interesting qualities that arise in diamond particles in the nanometer size. NDs are obtained in two different ways: by synthesis through the detonation of carbon-rich explosives (e.g., DNDs) or by milling synthetic diamond down to ten to a few hundred nanometers in size (e.g., milled HPHT NDs, and FNDs). These different ways of synthesizing nanodiamonds lead to diamond materials with different sizes, purities, and properties, which are discussed in more detail in **Chapter 1.**

The studies in this dissertation represent stepping stones toward the potential applications of nanodiamonds in a microbiological environment. The usefulness of FNDs as sensors for detecting free radical species in bacteria and DNDs potential as antibactericidal coating have been considered in this thesis.

Since the foremost step for using materials for biological and medical purposes is determining their lack of toxicity and biocompatibility, this thesis also aimed to systematically investigate the antimicrobial mechanism and biocompatibility of FNDs. FNDs are widely used as abrasives, optical or magnetic labels in drug delivery, or nanoscale sensing. FNDs are considered very biocompatible in mammalian cells. However, the situation looks different in bacteria, and the results are highly controversial. **Chapter 2** presents a short review of the published literature and a systematic experimental study of different strains, nanoparticle sizes, and surface chemistries. Most notably, particle aggregation behaviour and bacterial clumping are taken into consideration to explain reduced colony counts, which can be wrongly interpreted as a bactericidal effect. The experiments show no mechanism that can be linked to specific material properties but prove that aggregation and bacteriostatic effect of FNDs attachment play a significant role in the reported results.

Diamond magnetometry allows nanoscale magnetic resonance measurements by utilizing fluorescent defects in FNDs which convert magnetic resonance signals into optical signals. This thesis explores the utilization of relaxometry (also called T1 measurement), which is a specific type of magnetometry, to sense magnetic fields generated by free radicals in bacteria.

Free radical generation plays a key role in killing bacteria with antibiotics. However, radicals are short-lived and reactive and thus difficult to detect for the state of the art. **Chapter 3** explores the utilization of relaxometry (also called T1 measurement), which is a specific type of magnetometry, to sense magnetic fields generated by free radicals on the scale of single bacteria. We demonstrate that the radical generation in *Staphylococcus aureus* increases in the presence of UV irradiation and vancomycin and is dependent on the antibiotic's dose. With a method based on ensembles of nitrogen-vacancy (NV) centers in diamond, we were able to follow the radical formation near individual bacteria over the whole duration of the experiment to reveal the dynamics of radical generation. Using this new approach, we observed free radical concentrations within nanoscale voxels around the diamond particles and determined its exact timing depending on the antibiotic dose. Since changes in response to antibiotics emerge in only a few bacteria of the entire population, such a single-cell approach can prove highly valuable for research into drug resistance.

Bactericidal surfaces are in high demand for biomedical as well as industrial applications. In **Chapter 4**, we investigated if a new composite material, diamond coated black silicon surfaces (black diamond), is useful for this application. Black diamond is derived from black silicon, which is a silicon surface structured into nano-sized needles. These needles can pierce through bacteria on contact. Furthermore, the bactericidal and anti-bacterial properties of fluorine-terminated and hydrogen-terminated black diamonds with black silicon and flat surfaces of diamond (on silicon) with the same termination are compared in **Chapter 4**. While Gram-negative bacteria were investigated in previous studies, in this study we evaluated the ability to repel and kill Gram-positive *S. aureus* and *S epidermidis,* which have a thicker cell wall and are more mechanically robust. We evaluated the short-term 1 h initial adhesion as well as long-term 24 h biofilm formation. We found that the number of bacteria that initially adhered to the fluorine-terminated black diamonds surface was significantly reduced and had the highest dead bacterial ratio compared to fluorine-terminated flat, black silicon surfaces and hydrogen-terminated surfaces, respectively. Biofilm formation after 24 hours showed that while all surfaces outperform glass over the long-term (24 h), diamond coated surfaces of fluorine and hydrogen-terminated surfaces significantly inhibit biofilm formation. In conclusion, fluorine and hydrogen diamond-coated surfaces with and without nano-needles have repelling, bactericidal and biofilm-inhibiting effects on Gram-positive bacterial strains and are promising antimicrobial surfaces.

Finally, in **Chapter 5**, the findings of this thesis are discussed in the light of ongoing research and conclude the outlines of future NDs application in the microbiology field.